REMARKS

The specification has been carefully reviewed and amended to correct typographical and clerical errors and to conform to the requirements regarding an application. The formulae for Formula I and Formula III have been amended to provide consistency to the symbols and the definition of the symbols used therein. The name of the culture deposit and the address thereof has been inserted in the specification at Page 6, lines 12-13 as amended. Since the specification was amended extensively, a substitute specification wherein the changes are marked is attached.

Claim 1 has been amended to define clearly Formula I and Formula III and to specify that the reaction is carried out using an enzyme or whole cell or cell extract of *Klyurvera Cirtophila.* Formula I and Formula III are now clearly defined. Claims 2 and 3 are cancelled as being redundant. Support for the amendment is found in originally filed claims 1-3 and Examples 1-3 on pages 5 - 6 of the specification.

No new matter is introduced. Entry of the amended specification and the claims is requested.

RESPONSE

Name of Cuture Deposit and address

The Examiner required the Applicant to supply the complete name and address of the ATCC. This has been provided now in the specification as amended at Page 6.

Rejection Under 35 U.S.C. §112

The Examiner rejected claims 1-15 under §112, second paragraph, for being indefinite in that the definitions for X and Y-Z are inconsistent for Formula I and Formula III; the designation of the compound Formula I as ±2-aza-bicyclo[2,2,1]hept-5-en-3-one now conforms with the formula presented for Formula I.

All use of parenthesis, dashes for bonds, and Ph in the claim 1 have now been eliminated. Claims 2-4 are cancelled. The objections to claims 1-4 are now moot.

Claims 1-14 were rejected as incomplete for not reciting a recovery step. Reconsideration of the rejection on this basis is requested for the following reason. In claim 1, the enantiomer specified as Formula III is selectively obtained and recovered by the process steps of extraction with an organic solvent, separating the organic layer and

removing the organic solvent. Claims 5-14 depends on claim 1 and further specified the buffer for the reaction, the organic solvents for the extraction step. It is believed that claim 1 is directed to a complete process with a recovery step. Therefore, the rejection on this basis should be withdrawn.

Rejection Under 35 U.S.C. §102

Claims 1-2 and 5 were rejected as anticipated by Dawson et al. US 6,340,587.

As amended, claims 1, 5 are directed to a process for obtaining an optically active enantiomer of Formula III by the use of an enzyme, an extract or the whole cell of *Klyuvera Citrophila.* Dawson et al describes a process for obtaining an optically active enantiomer of Formula III by the use of an acyclase enzyme, Savinase. Dawson et al. suggested that the acyclase may be obtained from microbacteria: *Subtilisin carlsberg, Bacillus sp., Humicola lanuginosa, Aspergillus sp.*

The processes described in Dawson et al. all employed savinase as the acyclase. In Example 2, the process include the following steps:

1. Reacting an N protected racemic compound, ± tert butyl 3-oxo-2-azabicyclo[2,2,1]hept-5-en-2 carboxylate wherein the protected group was carboxylate with a hydrolytic enzyme including Savinase in a mixture of tetrahydrofuran and a phosphate buffer, pH 8.0, at 30°C.

2. Increasing the pH to 9.0 followed by extraction with cyclohexane.

In Example 3, the steps are:

1. reacting ± tert butyl 3-oxo-2-azabicyclo[2,2,1]hept-5-en-2 carboxylate in tetrahydrofuran, with a suspension of sodium borohydrade in methanol, at 20°C for a total of 20 hours;

2. Adding hydrochloric acid and then toluene to extract the enantiomer, (1R, 4S) (4hydroxymethyl)-cyclopent-2-en-1-yl carbamic acid.

Dawson et al. also indicated that significant non-enzymatic hydrolysis of the substrate takes place if tetrahydrofuran was omitted from the reaction mixtures.

The enzyme used in the claimed process of the present invention is that from *Klyuvera Citrophila.* This is not described or taught by Dawson et al.

Dawson et al. also indicated that significant non-enzymatic hydrolysis of the substrate takes place if tetrahydrofuran was omitted from the reaction mixtures.

The enzyme used in the claimed process of the present invention is that from *Klyuvera Citrophila.* This is not described or taught by Dawson et al.

For anticipation to be found, each and every element of the claimed invention must be found within the four corners of the reference. Since, Dawson et al. does not teach or describe an enzyme from *Klyuvera Citrophila,* anticipation cannot be found.

Rejection Under 35 U.S.C. §103

Claims 1-2, and 5-15 were rejected as being obvious in view of a combination of the teachings or Dawson et al. taken with Bernegger-Egli et al and Evans et al.

Claim 1 and the claims dependent thereon are amended. As amended claim 1 incorporates the limitations of claims 3 and 4, which were indicated as allowable.

CONCLUSION

It is believed based on the indication of allowability of claims 3 and 4 that the claims as amended are allowable. An early allowance is requested.

Respectfully Submitted,

Date: November 13, 2003

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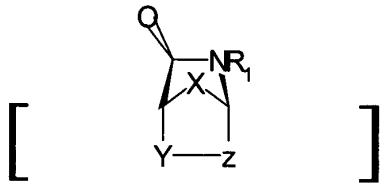
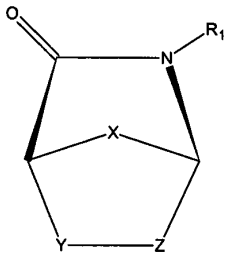
PROCESS FOR THE PREPARATION OF OPTICALLY ACTIVE AZABICYCLO HEPTANONE DERIVATIVES

Field of the ~~invention~~ Invention

The present invention relates to a process for the preparation of an optically active azabicyclo heptanone derivative of general ~~formula~~ Formula III

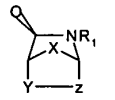


Formula III



Formula III

wherein R_1=H, X=CH_2, Y-Z = -CH=CH-, from a racemic mixture of the γ-lactams of ~~formula~~ Formula (I)



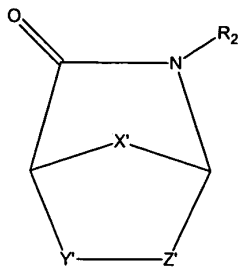
Formula I

Wherein R_1=H, COR_2 (R_2=C_{1-4} alkyl,C_{1-4} alkoxy,aryl,aryloxy)

X=O,CHR_3 (R_3=F,OH,Br,H)

Y-Z=-CH=CH-,CH_2-CH_2,-CH(O)-CH(O)-

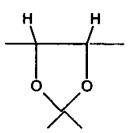
-CH(R4)-CH_2-(R_4=Br,OH,$PhCH_2O$,F,N_3)



Formula I

wherein R_2 is H or COR_3, (R_3 is C_{1-4} alkyoxy, aryl or aryloxy),

X' is O or CHR_4 (R_4 is F, OH, Br, or H),

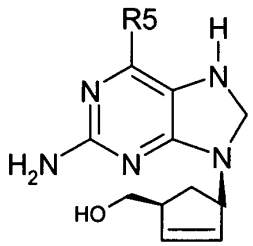
Y'-Z' is CH=CH, 

or -$CH(R_6)CH_2$- (R_6 = Br, OH, $PhCH_2O$, or N_3.

The present invention also relates to a process for the preparation of an optically active azabicyclo heptanone derivative of general ~~formula~~ Formula III wherein R_1= H, X'= CH_2, Y'-Z'= CH = CH_2 is useful as an intermediate in the synthesis of antiviral agents.

Background of the invention

Carbocyclic analogues of purines are known as antiviral and anti neoplastic agents. For example the compound of ~~formula~~ Formula ~~(II)~~ is described as having potent activity against human immunodeficiency virus (HIV) and Hepatitis B virus (HBV) (EP 0434450).



Formula - II

wherein R_5 =Cyclopropylamino, or N-Cyclopropyl, N-Methylamine.

Prior art discloses the preparation of 9-substituted-2-amino purines starting from a pyrimidine compound, coupling with enantiomerically pure sugar/carbocyclic analogues residue and cyclization to form the imidazole ring followed by introduction of suitable 6-substituent (PCT/GB95/00225). Carbovir and known analogues are prepared from the known γ-lactam (Vince lactam) 2-azabicyclo[2,2,1]hept-5-en-3-one (Formula I) wherein X' is $-CH_2$, -Y'-Z'- is –CH=CH- and R_{12} is H.

Prior art indicates that the final product or any intermediate or starting material may be resolved by known methods or the racemic mixture of the product may be enzymatically converted to chirally pure compound. The γ-lactam can be prepared by reacting cyclopentadiene with tosylcyanide,(Vince J. Org. Chem. 1978, 43, 2311).

There are several synthetic pathways where chemical resolution into the enantiomer has been effected but the enzymatic resolution of γ-lactam will be the most economical commercial process. γ-lactamase methodology has been reported based on enantio-complementary biotransformation. Enzymatic resolution of bicyclic lactam using whole cell cultures ENZA1 and ENZA2 has been reported to give both the optical forms of lactam (S.V. Teylor, J.C.S. Chem. Comm., 1121, 1990, Tet. Assy., 4, 1117-1128). The detailed process has been described in patent (EP 0424064). The racemic lactam was treated with ENZA-1/2 cell free extract at 30°C with shaking for 14 days. The crude (+)/ (-) lactam was isolated by extraction with dichloromethane and purified by column chromatography on silica gel. ~~The~~ to provide the (+) lactam ~~obtained~~ with 88% optical purity, ee, and the (-) lactam with 98% ee, optical purity .

Enzymatic resolution of N-Acyl bicyclic lactam using acylase has been described in patent (PCT/EP99/04814) in 31% yield with 98% ee. The conversion of the optically active N-Acetyl-lactam to(+) /(-) lactam is tedious.

The prior art methods to the cyclopentane moiety of carbocyclic nucleosides starting from non-carbohydrate synthons or readily available meso compounds generally involve a number of steps, which are often difficult to perform and provide poor yields, making the practical scale-up of these processes difficult and uneconomical.

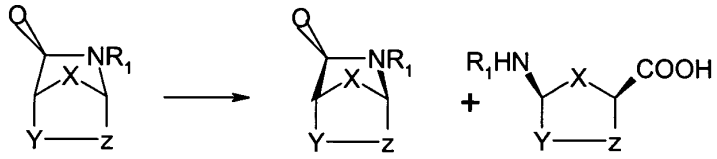
Objects of the invention

The main object of the present invention is to provide a process for the preparation of an optically active azabicyclo heptanone derivative which obviates the drawbacks of the ~~present invention~~ prior art processes and use cheaper and easily available microbial whole cell enzyme.

It is another object of the invention to provide a process for the preparation of (-) 2-Azabicyclo[2,2,1]-hept-5-ene-3-one ~~formula (III)~~ Formula III, which is economical and efficient.

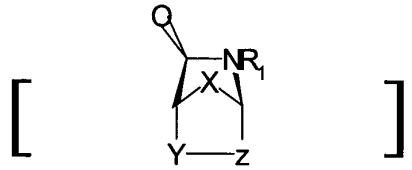
Summary of the invention

The present invention provides a process for the preparation of optically active azabicyclo heptanone derivatives using lactamases that will react with racemic γ-lactam of ~~formula (I)~~ Formula I to give a single enantiomer of lactam (III) and the corresponding ring opened compound of formula (IV) in an enantiomerically pure form.

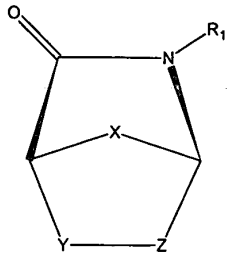


I III(-) IV(+)

Accordingly, the present invention provides a process for the preparation of (-)2-Azabicyclo[2,2,1]-hept-5-ene-3-one ~~formula(III)~~ Formula III wherein R_1 = H, X = CH_2, Y-Z = -CH=CH-,

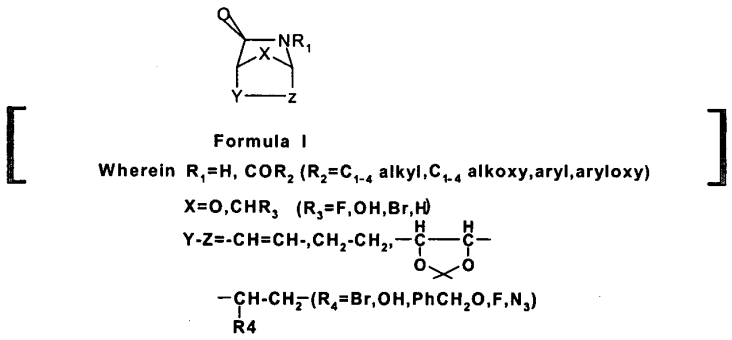


Formula III

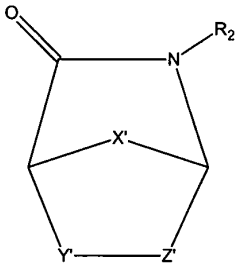


Formula III

which comprises reacting a racemic mixture of a compound of ~~formula (I)~~ Formula I:



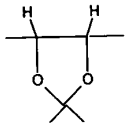
Formula I



Formula I

wherein R_2 is H or COR_3, (R_3 is C_{1-4} alkyoxy, aryl or aryloxy),

X' is O or CHR_4 (R_4 is F, OH, Br, or H),

Y'-Z' is CH=CH, 

or $-CH(R_6)CH_2-$ (R_6 = Br, OH, $PhCH_2O$, or N_3,

with an enzyme, a lactamase or the whole cells of a microorganism in a buffer containing organic solvent at temperature ranging between 25-30°C for a period ranging from 14 to 24 hr., extracting the mixture into an organic solvent, separating the organic layer and removing the organic solvent to obtain the product.

In one embodiment of the invention the microorganisms or enzymes used are selected from ~~the~~ *Bacillus, Klyuvera* ~~and~~ or *Eschericha*.

In another embodiment of the invention the whole cell is obtained from growing a culture of *Klyuvera Citrophila*, ATCC No.21285 (American Tissue and Type Culture, Box 1549, Manassas, VA 20108), in a conventional culture medium.

In another embodiment of the invention, the cell extract or enzyme used comprises an enzyme or a cell extract from *Klyuvera sp.* (ATCC No.21285).

In another embodiment of the invention the buffer used is selected from the group consisting of phosphate buffer (0.05 M – 0.1 M, 6-8 pH), citrate buffer (0.05 M – 0.1 M 6-7.5 pH) and Trisbuffer (0.05M- 0.2M, 7-8 pH).

In another embodiment of the invention the buffer used comprises phosphate buffer (0.2 M, 7.4 pH).

In another embodiment of the invention the organic solvent used for the reaction along with buffer is selected from the group consisting of alcohols, alkyl acetates, ketones and sulfoxides.

In a further embodiment of the invention, the organic solvent is selected from the group consisting of methanol, ethanol, butanol, ethyl acetate, acetone, dimethyl sulfoxide and dimethylformamide.

In a further embodiment of the invention, the organic solvent comprises acetone.

In another embodiment of the invention the percent of organic solvent used for the reaction along with buffer is in the range of 5% to 50% (v/v).

In another embodiment of the invention the percent of organic solvent used for the reaction along with buffer comprises is 10 %(v/v).

In another embodiment of the invention the solvent used for extraction comprises a chlorinated solvent selected from the group consisting of chloroform, ethylene dichloride, methylene dichloride and an alkyl acetate.

In another embodiment of the invention, the alkyl acetate used as the solvent for extraction comprises ethyl acetate.

In another embodiment of the invention the solvent used for extraction comprises methylene chloride.

In a feature of the invention the chemical yield (-) 2-Azabicyclo[2,2,1]-hept-5-ene-3-one is 39.3% and the optical purity is 98%.

Detailed description of the invention

The process of the present invention is highly efficient ~~and~~ with maximizes cost effectiveness by a fast resolution process to provide (-) lactam enantiomer, an important starting material for the production of the anti HIV agent (-) Carbovir and Abacavir.

The process of the present invention is described herein below with references to the following examples, which are illustrative only and should not be construed to limit the scope of the present invention in any manner.

Example 1

This example describes a general procedure for ~~cell~~ a biomass preparation ~~)~~ (using whole cell ~~)~~ or an enzyme pre-inoculum (5-10 ml) ~~was~~ prepared by growing a microorganism in a medium containing a yeast extract (0.5%), peptone (1%), sodium chloride (0.2%), sodium glutamate (0.5%) and phenyl acetic acid (15 mM) at pH 7.2-7.3 for 24 hr with shaking at 150 rpm. 300 ml of the above mentioned growth medium ~~This~~ was subsequently transferred to a 1 liter ~~Lt.~~ flask ~~containing 300 ml above mentioned growth medium~~ and incubated at 28-30°C for 24 hours on a rotary shakers (150 rpm). The biomass

of grown cells were separated by centrifuge and washed with phosphate buffer pH 6.8. ~~and~~ The separated biomass or cell mass was used for the reaction described in Example 2.

Example 2

The ~~General~~ general procedure for the enantioselectsive hydrolysis of (±)-2-azabicyclo (2,2,1) hept-5-en-3-one, (Vince's lactam) (1)~~.~~ is as follows:

0.1 g (0.00092 mole parts) of (±)-2-azabicyclo[2,2,1]-hept-5-en-3-one (1) was suspended in phosphate buffer (5 parts) and 50 mg of the wet ~~cell~~ biomass of culture (ATCC No.21285) was added. ~~and~~ The mixture was kept stirring for 72 hr. The cell mass was removed by filtering through celite and the filtrate was extracted with dichloromethane (5 x 10 parts). ~~Concentration~~ Removal of the solvent gave optically active Formula III in 31.8% chemical yield, and 58.2% ee.

Example 3

~~General~~ The general procedure for the enantioselective hydrolysis of (±)-2-azabicyclo(2,2,1)hept-5-en-3-one, using the cell mass obtained from culture (ATCC No.21285) was followed. 0.1 g (0.00092 mole parts) of the (±) racemic mixture was suspended in phosphate buffer (5 parts) and ~~different~~ an amount of the cell mass ~~(~~as indicated in Table 1~~)~~ was added. The mixture was ~~and~~ kept stirring for 24 hrs. The cell mass was then removed by filtering through celite and the filtrate was extracted with dichloromethane (5x10 parts). ~~Concentration~~ Removal of the solvent gave optically active Formula III. The results are summarized in Table 1.

Table 1

Sr.No.	Cells Wet/Wt.%	Chemical Yield	Ratio R:S	ee %
1.	5	39.1	46.36 : 53.64	7.29
2.	10	33.2	45.15 : 54.85	9.71
3.	20	31.4	36.70 : 63.30	26.61
4.	30	33.0	27.43 : 72.57	45.14
5.	40	34.2	25.71 : 74.29	48.58
6.	50	30.5	21.23 : 78.77	57.55

Example 4

~~General~~ The general procedure for enantioselective hydrolysis of (±)2-azabicyclo(2,2,1)hept-5-en-3-one, using the cell mass from culture (ATCC No.21285) was followed. 0.2 g (0.00184 mole parts) of (±) was suspended in phosphate buffer and an organic solvent,(as indicated in Table 2) 10 parts. 0.1 gm of wet cell mass was added and kept stirring 24 hrs. The cell mass was removed by filtering through celite and the filtrate was extracted with dichloromethane (5 x 10 parts). ~~Concentration~~ Removal of the solvent gave optically active Formula III. The results are summarized in Table 2.

Table 2

Sr.No.	Organic Solvent	Chemical Yield	Ratio R:S	ee %
1.	Ethyl acetate	27.1	13.39 : 86.61	73.22
2.	Methanol	25.5	14.90 : 85.10	70.20
3.	Ethanol	33.1	9.27 : 90. 73	81.46
4.	Acetone	44.2	9.69 : 90.31	80.62
5.	Dimethyl sulfoxide	42.1	36.10 : 63.90	27.80

The ratio of phosphate buffer (0.2M, pH 7.4) to organic solvent is (9:1).

Example 5

~~General~~ The general procedure for enantioselective hydrolysis of (±)2-azabicyclo(2,2,1)hept-5-en-3-one, using the cell mass from culture (ATCC No.21285) was followed. 0.2 g (0.00184 mole parts) of (±) was suspended in phosphate buffer and acetone (as indicated in Table 2) 10 parts. 0.1 gm of wet cell mass was added and the mixture was kept stirring for 24 hrs. The cell mass was removed by filtering through celite and the filtrate was extracted with dichloromethane (5 x 10 parts). ~~Concentration~~ Removal of the solvent gave optically active Formula III. The results of different proportions of acetone are summarized in Table 3.

Table 3

Sr.No.	Buffer : Acetone(v/v)	Chemical Yield	Ratio R:S	ee %
1.	9.5 : 0.5	40.8	4.58 : 95.85	90.85
2.	9.0 : 1.0	41.2	9.69 : 90.31	80.62
3.	8.0 : 2.0	41.3	12.34 : 87.66	75.33
4.	5.0 : 5.0	Slow reaction	-	-

Example 6

~~General~~ The general procedure for the enantioselective hydrolysis of (±)2-azabicyclo(2,2,1)hept-5-en-3-one, using cell mass from culture (ATCC No.21285) was followed. 10.0 g (0.918 mole parts) of (±) was suspended in mixture of 475 parts of phosphate buffer and 25 parts of acetone in a 1 liter ~~Lt.~~ flask. Cell mass (wet. Weight ~ 5 parts) was added and the reaction mixture was stirred at room temperature (28±1°C). After completion of the reaction (monitored by chiral HPLC) the reaction mixture was centrifuged in order to remove the cell mass. ~~and~~ The supernatent liquid was extracted using a continuous extractor by dichloromethane. On evaporation of the solvent under reduced pressure, (1S, 4R) azabicyclo(2,2,1)hept-5-en-3-one III (3.93 gm) was obtained. ~~Crystallisation~~ Crystallization with a dichloromethane : ether mixture gave a product of 98% optical purity.